Deciphering pathological remodelling of the human cartilage extracellular matrix in osteoarthritis at the supramolecular level

Electronic Supplementary Material

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Content

S1. XPS data

S1.1. Analysis depth

XPS probes a thickness of only few nanometres. In fact, an electron photoemitted at depth z in a flat and smooth solid in a direction making the angle θ with the normal to the surface of the solid has a probability Q to leave the solid in this direction without energy loss: $Q = \exp(-z / \lambda \cos\theta)$ where $z / \cos\theta$ is the distance *d* travelled by the photoelectron in the solid and λ is the inelastic mean free path (IMFP), i.e. the average distance between inelastic collisions ($\lambda = -dd/dQ$). The IMFP depends on the kinetic energy of the photoemitted electron and on the properties of the matrix, particularly its density. The contribution to the intensity decreases exponentially with the depth: 95 % from a layer of thickness $3\lambda \cos\theta$ [1].

IMFP values were calculated according to Tanuma et al. [2] using the Quases program [3] using the parameters given in Table S1. These calculations lead to the values detailed in Table S2. The thickness probed by XPS for each cryo-sectioned sample is thus around 5.5 nm.

Table S1. Parameters used for the calculation of the electron inelastic mean free path (IMFP). (Abbreviations. Col: collagen, Agg: aggrecan, HA: hyaluronic acid and HC: hydrocarbon compounds)

Quases program input data	Col	Agg	HA	HC
Carbon quantity	12321	65800	14	1
Mw (g/mol)	289639	2421399	379	14
Carbon concentration (mol/g)	0.043	0.027	0.037	0.071
Density (g/cm^3)	1.4	1.5	1.5	0.9
Valence electrons	113043	173	148	6
C_C^{ECM} **(mol/cm^3)**	0.0596	0.0408	0.0554	0.0643
C^{ECM} **(mol/cm^3)**	**0.055**			

Table S2. Computation of the electron inelastic mean free path (IMFP).

Energy Gap (eV)		2	4	6	8	λ_{moy}^{Matrix}
Col	λ_C	3.3	3.4	3.6	4.0	3.6
	λ_N	3.0	3.2	3.4	3.7	3.3
Agg	λ_C	3.3	3.4	3.6	3.9	3.5
	λ_N	3.0	3.2	3.3	3.6	3.3
HA	λ_C	3.3	3.4	3.7	4.0	3.6
	λ_N	3.1	3.2	3.4	3.7	3.3
Others	λ_C	3.3	3.5	3.8	4.2	3.7
	λ_N	3.1	3.2	3.5	3.9	3.4
λ_{moy}^{Org} (nm)		3.2	3.3	3.5	3.9	3.5
$3\lambda_{moy}^{Org}$ (nm)		9.5	9.9	10.6	11.6	10.4
$3\lambda_{moy}^{Org}\cos(\theta)$ (nm)		5.0	5.2	5.6	6.1	5.5

S1.2. Spectral components and chemical functions

Table S3. Characteristics and assignment of XPS peaks components for human cartilage samples.

Peak	Binding energy (eV)	Functional groups	
C 1s	284.8 * †	**C**–(C,H)	hydrocarbon
	286.1 ± 0.2 †	**C**–(O,N)	alcohol, ether, ester, amide, amine
	287.5 ± 0.3 †	O–**C**–O	acetal, hemiacetal
		C–(**C**=O)–N	amide
	288.9 ± 0.2 †	C–(**C**=O)–O	carboxylic acid, ester
N 1s	399.8 ± 0.1	(C=O)–**N**H	amine / amide
	401.5 ± 0.4	C–(**N**H_3^+)	protonated amine
	402.4 ± 0.3	R_4–**N**$^+$	quaternary amine
S 2p	163.7 ± 0.3 §	R–**S**–**S**–R’	protein disulfide bond
	168.6 ± 0.3 §	**S**O_4^{2-}	sulfate

* Component fixed at 284.8 eV and used for referencing XPS peaks.

† Components imposed to have the same FWHM, found in the range of 1.1 to 1.6 eV.

§ Component $^3/_2$

S1.3. Elemental composition

Table S4. Elemental composition measured by XPS on treated cartilages. Molar concentrations over all elements except hydrogen. (bdl: below the detect limit)

Treatment	Analysed zone	C	N	O	S	Si	Zn	Na	Ca	P
NF	A	73.00	1.91	24.43	0.40	0.23	0.02	0.01	bdl	bdl
	B	71.09	6.62	20.72	1.06	0.22	bdl	0.24	0.05	bdl
	C	67.70	9.45	20.35	1.20	0.65	bdl	0.55	0.10	bdl
F	A	69.46	0.37	29.11	0.07	0.77	0.07	0.15	bdl	bdl
	B	70.39	2.56	25.87	0.27	0.65	0.03	0.21	0.02	bdl
	C	67.39	7.02	23.51	0.59	0.82	0.02	0.55	0.10	bdl
F – *Pa*	A	79.16	5.52	14.06	0.52	0.37	bdl	0.07	0.22	0.08
	B	80.42	4.25	13.54	0.50	1.00	bdl	0.05	0.22	0.02
	C	84.69	2.33	10.54	0.36	1.90	bdl	0.03	0.15	bdl
F – *d*	A	79.98	4.64	14.19	0.42	0.42	0.01	0.34	bdl	bdl
	B	86.03	1.70	11.23	0.18	0.69	0.04	0.13	bdl	bdl
	C	72.01	2.01	18.56	0.18	6.79	0.15	0.30	bdl	bdl
F – *d* – *us*	A	66.33	10.63	20.54	1.19	0.29	bdl	1.02	bdl	bdl
	B	67.28	8.38	21.18	1.40	0.91	0.03	0.82	bdl	bdl
	C	74.98	2.41	20.09	0.57	1.76	0.04	0.15	bdl	bdl

S2. Concentrations of elements and chemical functions expected for ECM main compounds

S2.1. Type II collagen

Table S5. Elemental composition of type II collagen, computed from amino acid amounts and composition.

amino acids	quantities	AMINO ACID					ALPHA CHAIN					TROPOCOLLAGEN				
		C	H	N	O	S	C	H	N	O	S	C	H	N	O	S
A	108	3	7	1	2		324	756	108	216		972	2268	324	648	
D	32	4	7	1	4		128	224	32	128		384	672	96	384	
E	55	5	9	1	4		275	495	55	220		825	1485	165	660	
F	15	9	11	1	2		135	165	15	30		405	495	45	90	
G	352	2	5	1	2		704	1760	352	704		2112	5280	1056	2112	
H	2	6	9	3	2		12	18	6	4		36	54	18	12	
I	11	6	13	1	2		66	143	11	22		198	429	33	66	
K	39	6	14	2	2		234	546	78	78		702	1638	234	234	
L	28	6	13	1	2		168	364	28	56		504	1092	84	168	
M	11	5	11	1	2	1	55	121	11	22	11	165	363	33	66	33
N	12	4	8	2	3		48	96	24	36		144	288	72	108	
P	200	5	9	1	2		1000	1800	200	400		3000	5400	600	1200	
HYP	29	5	10	1	3		145	290	29	87		435	870	87	261	
Q	41	5	10	2	3		205	410	82	123		615	1230	246	369	
R	54	6	14	4	2		324	756	216	108		972	2268	648	324	
S	29	3	7	1	3		87	203	29	87		261	609	87	261	
T	21	4	9	1	3		84	189	21	63		252	567	63	189	
V	19	5	11	1	2		95	209	19	38		285	627	57	114	
Y	2	9	11	1	3		18	22	2	6		54	66	6	18	
TOTAL	**1060**						**4107**	**8567**	**1318**	**2428**	**11**	**12321**	**25701**	**3954**	**7284**	**33**
1059 H_2O removed during peptide bond formation								2118		1059			6354		3177	
total number of each element							**4107**	**6449**	**1318**	**1369**	**11**	**12321**	**19347**	**3954**	**4107**	**33**
molar mass of each element (g/mol)							12.01	1.01	14.01	16.00	32.06	12.01	1.01	14.01	16.00	32.06
M_W (g/mol)							96546					289639				

Table S6. Chemical functions in type II collagen (triple-helix) computed from composition (Uniprot) and pooled according to the expected contribution in XPS peaks: C 1s, O 1s and N1s.

TROPOCOLLAGEN																		
amino acids	$\underline{C}H_3$	$\underline{C}H_2$	$\underline{C}H$	$\underline{C}\underline{C}C$	$\underline{C}$-O	$\underline{C}$-N	$\underline{C}$-S	N-$\underline{C}$=O, N-C-N, C=N	O=$\underline{C}$-OH	C_{tot}	(C$\underline{N}$)=O	C-$\underline{N}$(H,C)	N_{tot}	$\underline{O}$H	$\underline{O}$=C-OH	O=C-$\underline{O}$H	N-C=$\underline{O}$	O_{tot}
A	324					324		324		972	324		324				324	324
D		96				96		96	96	384	96		96		96	96	96	288
E		330				165		165	165	825	165		165		165	165	165	495
F		45	225	45		45		45		405	45		45				45	45
G						1056		1056		2112	1056		1056				1056	1056
H		6				18		12		36	6	12	18				6	6
I	66	33	33			33		33		198	33		33				33	33
K		351				234		117		702	117	117	234				117	117
L	168	84	84			84		84		504	84		84				84	84
M		33				33	66	33		165	33		33				33	33
N		36				36		72		144	72		72				72	72
P		1200				1200		600		3000	600		600				600	600
HYP		87			87	174		87		435	87		87	87			87	174
Q		246				123		246		615	246		246				246	246
R		324				324		324		972	162	486	648				162	162
S					87	87		87		261	87		87	87			87	174
T	63				63	63		63		252	63		63	63			63	126
V	114		57			57		57		285	57		57				57	57
Y		6	24	6	6	6		6		54	6		6	6			6	12
SUM	**735**	**2877**	**423**	**51**	**243**	**4158**	**66**	**3507**	**261**	**12321**	**3339**	**615**	**3954**	**243**	**261**	**261**	**3339**	**4104**
substraction of chemical ending								-3	3	**0**	-3	3	**0**		3	3	-3	**3**
TOTAL	735	2877	423	51	243	4158	66	3504	264	12321	3336	618	3954	243	264	264	3336	4107

S2.2. FACIT type IX collagen

Table S7. Elemental composition of type IX collagen, computed from amino acid amounts and composition.

amino acids	AMINO ACID					ALPHA CHAIN 1						per ALPHA CHAIN 2						per ALPHA CHAIN 3						TROPOCOLLAGEN				
	C	H	N	O	S	qties	C	H	N	O	S	qties	C	H	N	O	S	qties	C	H	N	O	S	C	H	N	O	S
A	3	7	1	2		47	141	329	47	94	0	42	126	294	42	84	0	45	135	315	45	90	0	402	938	134	268	0
C	3	7	1	2	1	11	33	77	11	22	11	4	12	28	4	8	4	6	18	42	6	12	6	63	147	21	42	21
D	4	7	1	4		39	156	273	39	156	0	24	96	168	24	96	0	28	112	196	28	112	0	364	637	91	364	0
E	5	9	1	4		47	235	423	47	188	0	31	155	279	31	124	0	32	160	288	32	128	0	550	990	110	440	0
F	9	11	1	2		22	198	242	22	44	0	4	36	44	4	8	0	4	36	44	4	8	0	270	330	30	60	0
G	2	5	1	2		217	434	1085	217	434	0	203	406	1015	203	406	0	210	420	1050	210	420	0	1260	3150	630	1260	0
H	6	9	3	2		8	48	72	24	16	0	8	48	72	24	16	0	4	24	36	12	8	0	120	180	60	40	0
I	6	13	1	2		36	216	468	36	72	0	23	138	299	23	46	0	14	84	182	14	28	0	438	949	73	146	0
K	6	14	2	2		42	252	588	84	84	0	38	228	532	76	76	0	29	174	406	58	58	0	654	1526	218	218	0
L	6	13	1	2		57	342	741	57	114	0	28	168	364	28	56	0	49	294	637	49	98	0	804	1742	134	268	0
M	5	11	1	2	1	12	60	132	12	24	12	11	55	121	11	22	11	6	30	66	6	12	6	145	319	29	58	29
N	4	8	2	3		20	80	160	40	60	0	6	24	48	12	18	0	5	20	40	10	15	0	124	248	62	93	0
P	5	9	1	2		153	765	1377	153	306	0	138	690	1242	138	276	0	137	685	1233	137	274	0	2140	3852	428	856	0
Q	5	10	2	3		39	195	390	78	117	0	34	170	340	68	102	0	25	125	250	50	75	0	490	980	196	294	0
R	6	14	4	2		54	324	756	216	108	0	27	162	378	108	54	0	32	192	448	128	64	0	678	1582	452	226	0
S	3	7	1	3		42	126	294	42	126	0	18	54	126	18	54	0	25	75	175	25	75	0	255	595	85	255	0
T	4	9	1	3		26	104	234	26	78	0	15	60	135	15	45	0	11	44	99	11	33	0	208	468	52	156	0
V	5	11	1	2		37	185	407	37	74	0	29	145	319	29	58	0	20	100	220	20	40	0	430	946	86	172	0
W	11	12	2	2		6	66	72	12	12	0		0	0	0	0	0		0	0	0	0	0	66	72	12	12	0
Y	9	11	1	3		6	54	66	6	18	0	6	54	66	6	18	0	2	18	22	2	6	0	126	154	14	42	0
TOTAL						921	4014	8186	1206	2147	23	689	2827	5870	864	1567	15	684	2746	5749	847	1556	12	9587	19805	2917	5270	50
H_2O removed during peptide bond formation						920		1840		920		688		1376		688		683		1366		683						
total number of each element							4014	6346	1206	1227	23		2827	4494	864	879	15		2746	4383	847	873	12	9587	19805	2917	5270	50
molar mass of each element (g/mol)							12.01	1.01	14.01	16.00	32.06		12.01	1.01	14.01	16.00	32.06		12.01	1.01	14.01	16.00	32.06	12.01	1.01	14.01	16.00	32.06
M_w (g/mol)							91870						65131						63616					261889				

Table S8. Chemical functions in type IX collagen computed from composition (Uniprot) and pooled according to the expected contribution in XPS peaks: C 1s, O 1s and N1s.

	TROPOCOLLAGEN																	
amino acids	$\underline{C}H_3$	$\underline{C}H_2$	$\underline{C}H$	$\underline{C}\underline{C}\underline{C}$	$\underline{C}$-O	$\underline{C}$-N	$\underline{C}$-S	N-$\underline{C}$=O, N-$\underline{C}$-N, C=N	O=$\underline{C}$-OH	C_{tot}	(C$\underline{N}$)=O	C-$\underline{N}$(H,C)	N_{tot}	$\underline{O}H$	$\underline{O}$=C-OH	O=C-$\underline{O}$H	N-C=$\underline{O}$	O_{tot}
A	134					134		134		402	134		134				134	134
C						21	21		21	63		21	21		21	21		42
D		91				91		91	91	364	91		91		91	91	91	273
E		220				110		110	110	550	110		110		110	110	110	330
F		30	150	30		30		30		270	30		30				30	30
G						630		630		1260	630		630				630	630
H		20				60		40		120	20	40	60				20	20
I	146	73	73			73		73		438	73		73				73	73
K		327				218		109		654	109	109	218				109	109
L	268	134	134			134		134		804	134		134				134	134
M		29				29	58	29		145	29		29				29	29
N		31				31		62		124	62		62				62	62
P		856				856		428		2140	428		428				428	428
Q		196				98		196		490	196		196				196	196
R		226				226		226		678	113	339	452				113	113
S					85	85		85		255	85		85	85			85	170
T	52				52	52		52		208	52		52	52			52	104
V	172		86			86		86		430	86		86				86	86
W		6	24	12		18			6	66		12	12		6	6		12
Y		14	56	14	14	14		14		126	14		14	14			14	28
SUM	772	2253	523	56	151	2996	79	2529	228	9587	2396	521	2917	151	228	228	2396	3003
substraction of chemical ending								-3	3	0	-3	3	0		3	3	-3	3
TOTAL	772	2253	523	56	151	2996	79	2526	231	9587	2393	524	2917	151	231	231	2393	3006

S2.3. Aggrecan

Table S9. Elemental composition of aggrecan.

molecules	quantities	C	H	N	O	S
CS	100	58800	88200	4200	58800	4200
KS	50	7000	11500	500	7000	500
PC	1	11374	17633	2979	3982	47
total number of each element		77174	117333	7679	69782	4747
molar mass of each element		12.01	1.01	14.01	16.00	32.06
M_w (g/mol)		2421399				

S2.3.1. Sulphated GAG

Table S10. Elemental composition of sulphated GAG.

molecules	quantities	C	H	N	O	S
CS	100	58800	88200	4200	58800	4200
KS	50	7000	11500	500	7000	500
total number of each element		**65800**	**99700**	**4700**	**65800**	**4700**
molar mass of each element		12.01	1.01	14.01	16.00	32.06
M_W (g/mol)		2160071				

Table S11. Chemical functions in sulphated GAG (chondroitin sulphate and keratan sulphate) computed from composition and pooled according to the expected contribution in XPS peaks : C 1s, O 1s and N1s.

MOLECULE																			
GAGs	$\underline{C}H_3$	$\underline{C}H_2$	$\underline{C}H$	$\underline{C}\underline{C}\underline{C}$	$\underline{C}$-O	$\underline{C}$-N	$\underline{C}$-S	N-$\underline{C}$=O, N-$\underline{C}$-N, $\underline{C}$=N	O=$\underline{C}$-OH	C_{tot}	(C$\underline{N}$)=O	C-$\underline{N}$(H,C)	N_{tot}	$\underline{O}$H	$\underline{O}$=C-OH	O=C-$\underline{O}$H	N-C=$\underline{O}$	S=$\underline{O}$, S-$\underline{O}$	O_{tot}
CS	42				336	42		126	42	588	42		42	294	42	42	42	168	588
KS	10				80	10		40	0	140	10		10	90			10	40	140

S2.3.2. Core protein

Table S12. Elemental composition of collagen, computed from amino acid amounts and compositions.

amino acids	quantities	AMINO ACID					CORE PROTEIN				
		C	H	N	O	S	C	H	N	O	S
A	178	3	7	1	2		534	1246	178	356	
C	34	3	7	1	2	1	102	238	34	68	34
D	114	4	7	1	4		456	798	114	456	
E	284	5	9	1	4		1420	2556	284	1136	
F	68	9	11	1	2		612	748	68	136	
G	309	2	5	1	2		618	1545	309	618	
H	33	6	9	3	2		198	297	99	66	
I	88	6	13	1	2		528	1144	88	176	
K	28	6	14	2	2		168	392	56	56	
L	195	6	13	1	2		1170	2535	195	390	
M	13	5	11	1	2	1	65	143	13	26	13
N	30	4	8	2	3		120	240	60	90	
P	231	5	9	1	2		1155	2079	231	462	
Q	63	5	10	2	3		315	630	126	189	
R	79	6	14	4	2		474	1106	316	158	
S	309	3	7	1	3		927	2163	309	927	
T	204	4	9	1	3		816	1836	204	612	
V	196	5	11	1	2		980	2156	196	392	
W	25	11	12	2	2		275	300	50	50	
Y	49	9	11	1	3		441	539	49	147	
TOTAL	**2530**						**11374**	**22691**	**2979**	**6511**	**47**
2529 H_2O removed during peptide bond formation								5058		2529	
total number of each element							**11374**	**17633**	**2979**	**3982**	**47**
molar mass of each element (g/mol)							12.01	1.01	14.01	16.00	32.06
M_W (g/mol)							261329				

Table S13. Chemical functions in core protein computed from composition (Uniprot) and pooled according to the expected contribution in XPS peaks : C 1s, O 1s and N1s.

CORE PROTEIN																		
amino acids	$\underline{C}H_3$	$\underline{C}H_2$	$\underline{C}H$	$\underline{C}\underline{C}\underline{C}$	$\underline{C}$-O	$\underline{C}$-N	$\underline{C}$-S	N-$\underline{C}$=O, N-C-N,C=N	O=$\underline{C}$-OH	C_{tot}	($\underline{C}$N)=O	C-$\underline{N}$(H,C)	N_{tot}	$\underline{O}$H	$\underline{O}$=C-OH	O=C-$\underline{O}$H	N-C=$\underline{O}$	O_{tot}
A	178					178		178		534	178		178	0	0	0	178	178
C						34	34	34		102	34		34	0	0	0	34	34
D		114				114		114	114	456	114		114	0	114	114	114	342
E		568				284		284	284	1420	284		284	0	284	284	284	852
F		68	340	68		68		68		612	68		68	0	0	0	68	68
G						309		309		618	309		309	0	0	0	309	309
H		33				99		66		198	33	66	99	0	0	0	33	33
I	176	88	88			88		88		528	88		88	0	0	0	88	88
K		84				56		28		168	28	28	56	0	0	0	28	28
L	390	195	195			195		195		1170	195		195	0	0	0	195	195
M		13				13	26	13		65	13		13	0	0	0	13	13
N		30				30		60		120	60		60	0	0	0	60	60
P		462				462		231		1155	231		231	0	0	0	231	231
Q		126				63		126		315	126		126	0	0	0	126	126
R		158				158		158		474	79	237	316	0	0	0	79	79
S					309	309		309		927	309		309	309	0	0	309	618
T	204				204	204		204		816	204		204	204	0	0	204	408
V	392		196			196		196		980	196		196	0	0	0	196	196
W		25	100	50		75		25		275	25	25	50	0	0	0	25	25
Y		49	196	49	49	49		49		441	49		49	49	0	0	49	98
SUM	**1340**	**2013**	**1115**	**167**	**562**	**2984**	**60**	**2735**	**398**	**11374**	**2623**	**356**	**2979**	**562**	**398**	**398**	**2623**	**3981**
substraction of chemical ending								-1	1	**0**	-1	1	**0**		1	1	-1	**1**
TOTAL	**1340**	**2013**	**1115**	**167**	**562**	**2984**	**60**	**2734**	**399**	**11374**	**2622**	**357**	**2979**	**562**	**399**	**399**	**2622**	**3982**

Table S14. Chemical functions in aggrecan computed from composition and pooled according to the expected contribution in XPS peaks: C 1s, O 1s and N1s.

AGGRECAN																		
molecules	$\underline{C}H_3$	$\underline{C}H_2$	$\underline{C}H$	$\underline{C}\underline{C}\underline{C}$	$\underline{C}$-O	$\underline{C}$-N	$\underline{C}$-S	N-$\underline{C}$=O, N-C-N, C=N	O=$\underline{C}$-OH	C_{tot}	($\underline{C}$N)=O	C-$\underline{N}$(H,C)	N_{tot}	$\underline{O}$H	$\underline{O}$=C-OH	O=C-$\underline{O}$H	N-C=$\underline{O}$	S=$\underline{O}$, S-$\underline{O}$	O_{tot}
CS	4200				33600	4200		12600	4200	58800	4200		4200	29400	4200	4200	4200	16800	58800
KS	500				4000	500		2000		7000	500		500	4500			500	2000	7000
PC	1340	2013	1115	167	562	2984	60	2734	399	11374	2622	357	2979	562	399	399	2622		3982
TOTAL	**6040**	**2013**	**1115**	**167**	**38162**	**7684**	**60**	**17334**	**4599**	**77174**	**7322**	**357**	**7679**	**34462**	**4599**	**4599**	**7322**	**18800**	**69782**

S2.4. Hyaluronic acid

Table S15. Elemental composition of hyaluronic acid.

	MONOMER				MOLECULE				
HYALURONIC ACID	C	H	N	O	degree of polymerization	C	H	N	O
	14	21	1	11	10000	140000	210000	10000	110000
molar mass of each element (g/mol)	12.01	1.01	14.01	16.00	M_W (g/mol)	3793180			

Table S16. Chemical functions in hyaluronic acid computed from composition and pooled according to the expected contribution in XPS peaks: C 1s, O 1s and N1s.

HYALURONIC ACID																			
GAG	$\underline{C}H_3$	$\underline{C}H_2$	$\underline{C}H$	$\underline{C}\underline{C}\underline{C}$	$\underline{C}$-O	$\underline{C}$-N	$\underline{C}$-S	N-$\underline{C}$=O, N-C-N, C=N	O=$\underline{C}$-OH	C_{tot}	($\underline{C}$N)=O	C-$\underline{N}$(H,C)	N_{tot}	$\underline{O}$H	$\underline{O}$=C-OH	O=C-$\underline{O}$H	N-C=$\underline{O}$	S=$\underline{O}$, S-$\underline{O}$	O_{tot}
HA	10000				80000	10000		30000	10000	140000	10000		10000	80000	10000	10000	10000		110000

S3. Molar concentration ratios computed on the basis of amino acid amount and composition of ECM main compounds

Table S17. Expected molar concentration ratios from XPS computed on the basis of amino acid amount and composition of type II collagen, aggrecan and hyaluronic acid.

COMPONENT	C/ N	C_{ox} / N	$C_{284.8}$ / N	$C_{287.5}$ / N	$C_{286.1}$ / C	C/ S_{ox}
	α	β	δ	-	γ_x	η
Col	3.12	2.08	1.03	0.89	0.36	-
Agg	-	-	-	2.26	0.59	16.42
HA	-	-	-	3.00	0.64	-

S4. OA samples scoring

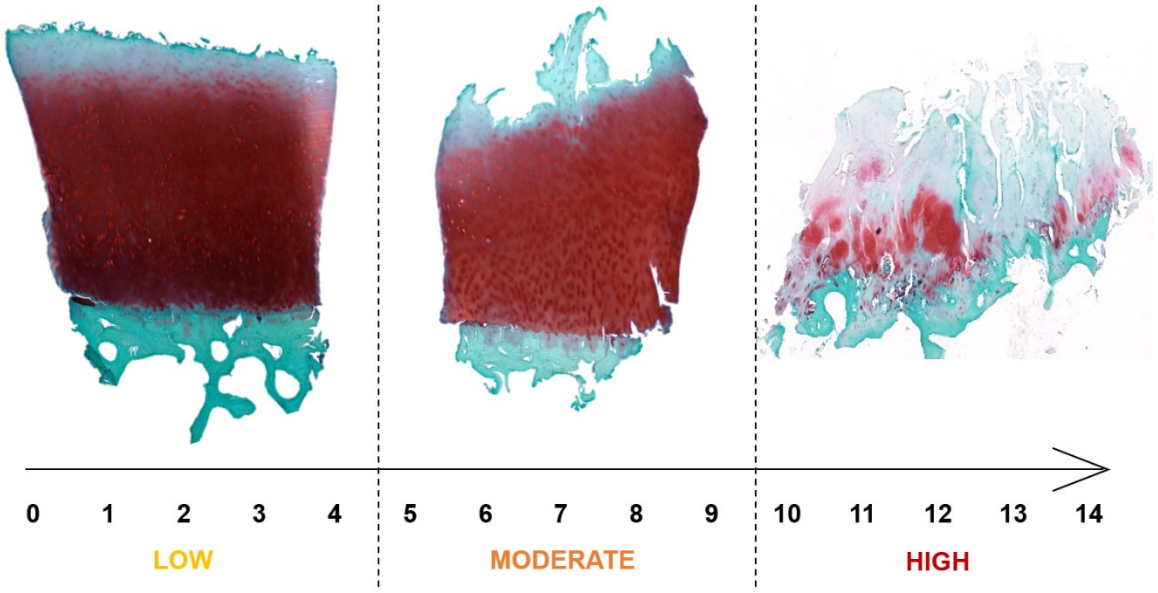


Figure S1. Evolution of the osteochondral junction due to OA. Tissues were stained with Safranin O / Fast Green. Degradation of articular cartilage was determined by the Mankin score on stained tissues. OA samples were then classified into groups with increasing OA severity: the group 'Low" (score 0 - 4), the group "Moderate" (score 5 - 9) and the group "High" (score 10 - 14).

S5. AFM data

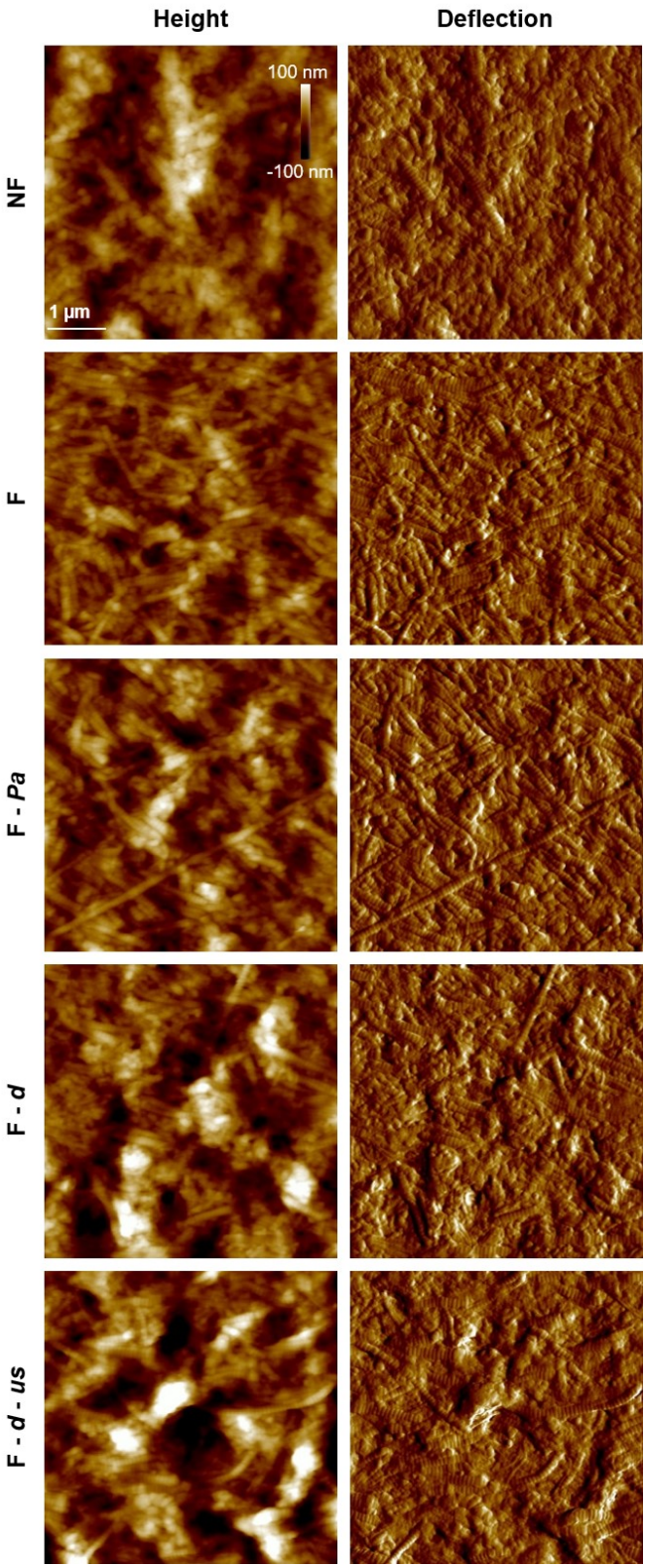


Figure S2. AFM height (left) and deflection (right) images recorded on human articular cartilage submitted to different treatments (see Table 1).

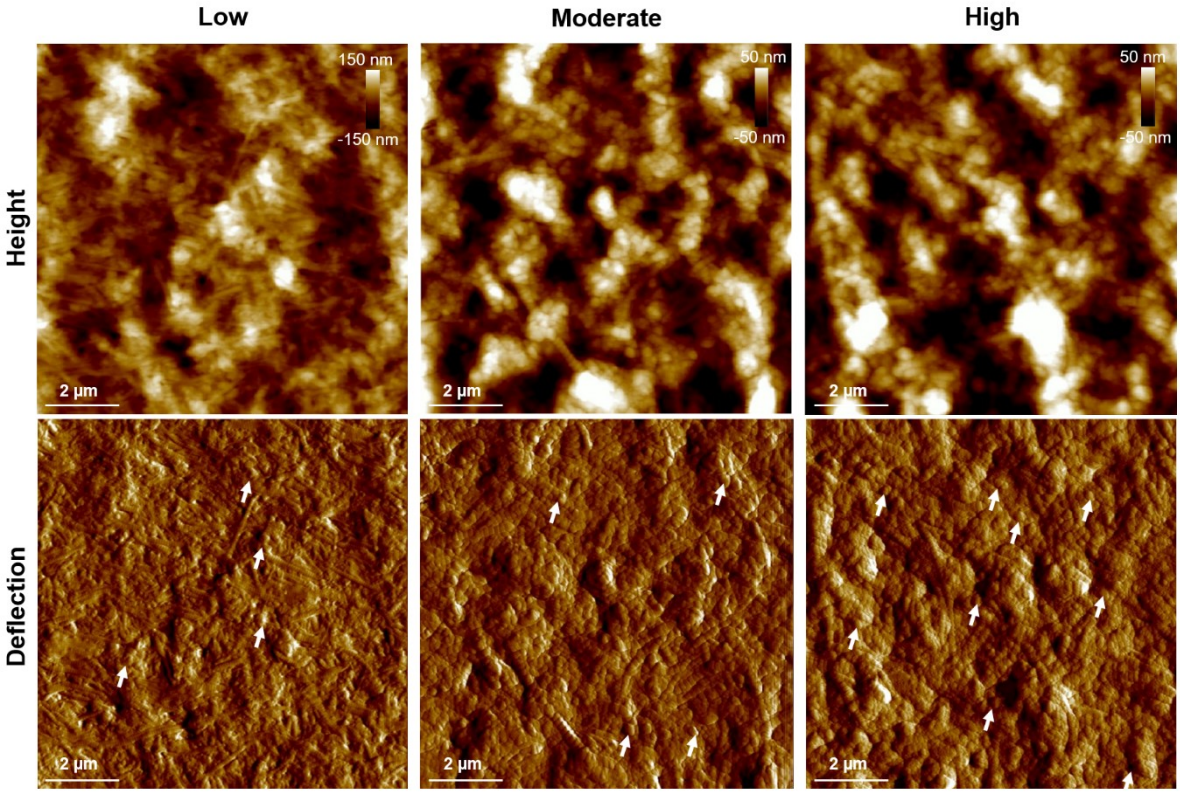


Figure S3. AFM height (top) and deflection (bottom) images recorded on OA cartilage ECM at low, moderate and high scores, showing the presence of spheroid-shaped particles (see arrows).

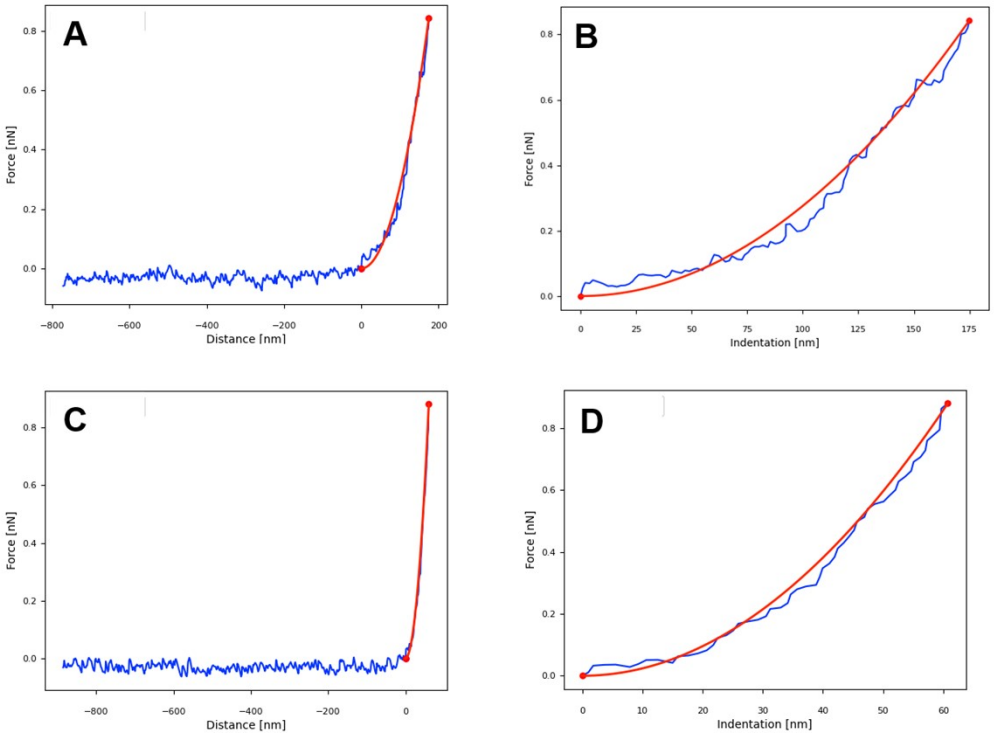


Figure S4. Typical (A, C) force-distance and (B, D) force-indentation curves recorded on cartilage ECM showing different stiffness. Experimental approach curves and the corresponding fitting curves, obtained using the Bilodeau model, are shown, respectively, in blue and in red.

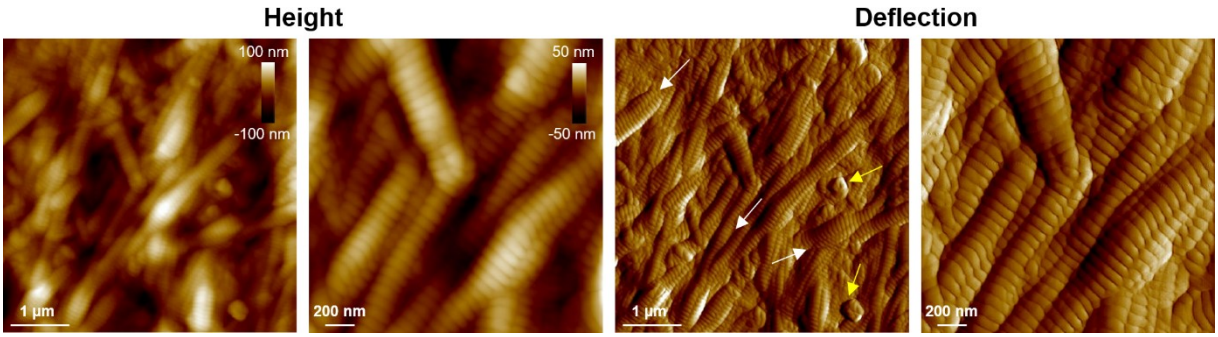


Figure S5. AFM height and deflection images (as indicated) recorded on a healthy cartilage after enzymatic hyaluronidase/collagenase treatment. Lateral fusion of collagen fibrils and spheroid-shaped nanostructures are indicated by white and yellow arrows, respectively.

S6. XPS data

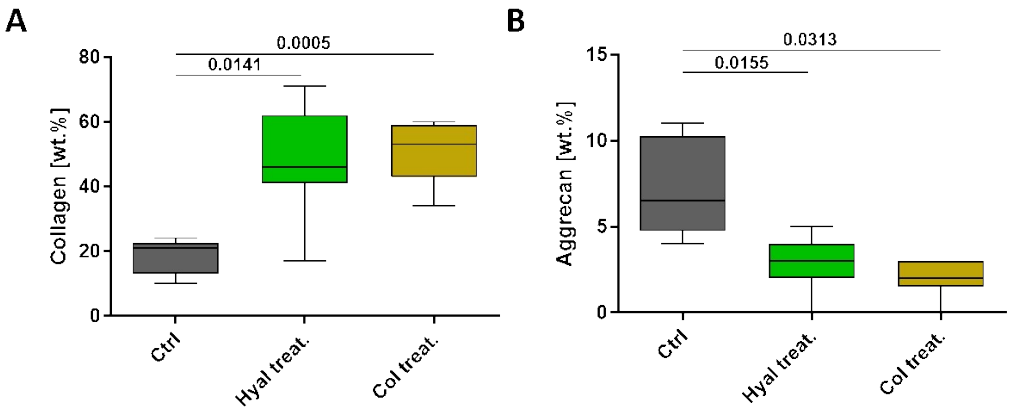


Figure S6. Evolution of the weight percentages (wt.%) of (A) collagen and (B) aggrecan prior to and after hyaluronidase (Hyal treat.) and collagenase (Col treat.) treatments.

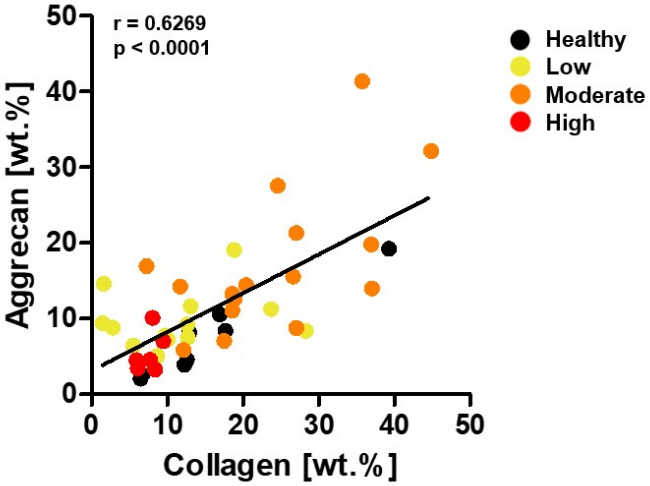


Figure S7. Correlation between the amount of collagen and aggrecan with a discrimination by histopathological OA severity. [Spearman correlation of 0.6269 ; p-value < 0.0001].

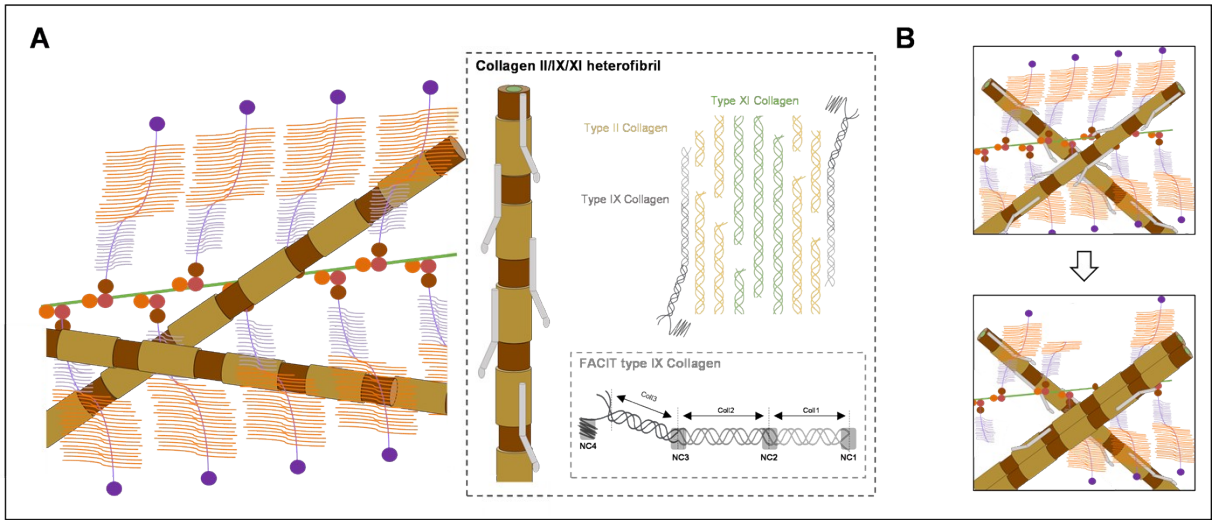
S7. Nanomechanical properties

(see Table 18)

Table S18. Nanomechanical properties measured by AFM on cartilage tissues from the literature.[4–9]

Ref	TISSUE							SCORING	AFM MODE			RESULTS
	species	joint	localisation within joint	sample preparation (shape, treatment, storage and cutting)					tip	parameters	localisation and direction	
Tang et al., Soft Materials, 2014	Human	hip; knee	NI	piece of 2mm²	collagen extraction	H_2O w/ 2%SDS, 4°C	/	/	radius < 10nm	k = 8 or 42 N.m^{-1}	*NI : extracted collagen fibrils*	Stiffening with OA
Shao et al., RSC Adv., 2014	Human	knee	NI	piece of 2mm²	/	OCT, -24°C	sectioned parallelly to surface (5-20 µm)	Outerbridge	nominal tip radius less than 7 nm	k=20.36 nN.nm^{-1}	cartilage surface; perpendicular	Stiffening with OA severity
Wilusz et al., Osteo. and Cart.,2013	Human	knee	femoral condyle	explant (8mm)	/	OCT, -20°C	sectioned perpendicularly to surface	Collin	microindentation, borosilicate glass spheres (5 µm nominal diameter)	k=7.5 N.m^{-1}, v=15 µm.s^{-1}, F=750 nN	cartilage depth; parallel	Softening with OA severity
Wen, Osteo. and Cart., 2012	Human	hip; knee	femoral head, lateral plateau tibial	osteochondral plug (5mm)	/	OCT, -20°C	sectioned parallelly to surface (50 mm)	Outerbridge	curvature radius of around 10 nm	k = 28–91 N.m^{-1}	cartilage different depth, perpendicular	Stiffening with OA severity
Stolz et al., Nat. Nano, 2009	Human	hip, knee	NI	osteochondral plug (2mm)	/	PBS, ice	/	Outerbridge	micro and nano-indentation (30nm and 150nm radius)	k=0.06 N.m^{-1}, f=3Hz, F=1.8 nN	cartilage surface; perpendicular	Softening with OA severity only with nano-indentation
Danalache et al., Osteo. and Cart.,2019	Human	knee	Femoral chondyle	explant	/	OCT	sectioned parallelly to surface (35 µm)	SCSO	a polymeric microsphere (25 µm in diameter)	k = 7.4 N.m^{-1} v = 5 µm.s^{-1} F= 300nN	cartilage surface; perpendicular	Softening with OA severity

S8. Collagen II/IX/XI heterofibril



Scheme S1. (A) Schematic illustration of the Collagen II/IX/XI hetero-fibril constituents and their organization (Abbreviations. FACIT: Fibril Associated Collagens with Interrupted Triple helixes, COL1/COL2/COL3: triple-helical domains). (B) Schematic illustration of the reorganization in the extracellular matrix of cartilage during osteoarthritis.

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